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THE MODE OF ACTION OF SOME NON-SPECIFIC ACETYLCHOLINE ANTAGONISTS

BY

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Antihistamines such as 2(*N*-benzyl-*N*-phenyl-aminomethyl)-iminazoline hydrochloride (antazoline hydrochloride) and *N*-dimethyl-aminoethyl benzhydryl ether hydrochloride (diphenhydramine hydrochloride), have been shown to possess many side-effects (Dutta, 1949), including local anaesthetic effects, antagonism of the depressant action of acetylcholine on the heart, depression of the maximal rate at which the isolated rabbit auricles will respond to electrical stimulation, antagonism of acetylcholine on isolated frog rectus abdominis muscle, and depression of the contraction of the nictitating membrane in response to preganglionic stimulation. Often, the concentrations of antihistamines required to produce such effects considerably exceed those necessary to produce the more specific effects against histamine, so that their modes of action are obscure. Marshall (1955a) claimed to have shown that the antagonism between many antihistamines and histamine on the guinea-pig ileum is competitive, although the criteria upon which competition was judged are not in entire agreement with those adopted in this paper.

A report is presented upon a preliminary investigation into the mode of action of two series of antihistamines as antagonists of acetylcholine (ACh) on the isolated guinea-pig ileum.

METHODS

The structure of the two series of compounds used in this work appear in Tables I and II.

The lever used throughout this work was constructed according to the dimensions suggested by Schild (1947), and was not allowed to move more than 30° from the horizontal. These precautions ensure that all measurements of the heights of excursions from the kymograph paper are linearly related to the actual shortening of the muscle.

Measurement of Anti-Acetylcholine Activity.—In order to determine the anti-ACh activity of these compounds, pA_2 and pA_{10} values (Schild, 1947) were measured against ACh on the isolated guinea-pig ileum. The tissue was suspended in an oxygenated Tyrode solution which was contained in the 2.0 ml. bath of an automatic assay apparatus of the type described by Boura, Mongar, and Schild (1954). Doses of ACh bromide (usually 0.0125 μg. base/ml. as basic dose) were added every 2 min. and antagonists were allowed to remain in contact for 10 min., represented by 5 complete cycles of the apparatus. pA_2 and pA_{10} values for each compound were not always determined on the same sample of ileum. Samples of ileum which did not recover fully from the effects of the antagonists were discarded, and fresh samples were used for the next test (Schild, 1947). Four determinations of pA_2 and four of pA_{10} were carried out for each substance using samples of intestine from different guinea-pigs.

Concentration-action Curves.—In order to determine the validity of the pA method as applied to the active-drug/antagonist systems studied, concentration-action curves were plotted for the active substance, ACh or histamine, first alone and then after equilibration to a fixed concentration of antagonist (Schild, 1949). The importance of such curves is discussed later in this paper, and the experimental procedure adopted to obtain them was as follows.

Fresh guinea-pig ileum samples were set up in the automatic isolated organ bath in the normal manner, and were stimulated every two minutes by a constant submaximal dose of acetylcholine bromide, until three consecutive contractions of approximately equal height were obtained. Usually between ten and twenty stimulations were required before this state was reached. Either 0.25 μg. or 0.05 μg. ACh/ml. was used, choice depending upon the sensitivity of the preparation. When constant responses had been obtained, six graded concentrations of ACh were administered, set up in the automatic bath in two groups of three concentrations. Within each group, the doses were administered in random order; three responses to each dose were obtained.

The concentrations of ACh usually employed and their normal groupings were, in μg./ml.:

(a) 0.125, 0.05, 0.10.

(b) 0.025, 0.075, 0.15.

At the end of each set, a single contraction was elicited by the addition of 1.0 μg. ACh/ml. This concentration of ACh always produced a maximal contraction of the muscle in the absence of antagonist, although sometimes

this was achieved with only 0.5 μg./ml. Maximal or near maximal doses had a detrimental effect upon the muscle, causing diminished responses to subsequently administered sub-maximal doses. Care was taken to eliminate any errors resulting from this, by ascertaining that the responses to lower concentrations of spasmogen were unaffected after maximal or near maximal doses. When this was not so, the muscle was allowed to recover before proceeding with the experiment.

Data for the curves in the presence of antagonist were obtained in the following manner. The wash fluid was changed from normal Tyrode to Tyrode containing the antagonist in the test concentration, which was usually in the region of the pA_{10} level. The gut was stimulated by a higher dose of ACh, usually 0.5 μg./ml., containing the test concentration of antagonist, until equilibrium between the antagonist and the receptors was obtained. This was indicated by a levelling out of the heights of contraction, which, before equilibrium, had tended to decrease with time of contact between antagonist and muscle. Equilibrium was usually reached within 15 min.

At this stage, the preparation was stimulated by six doses of ACh bromide, added in two groups of three doses as before, each individual dose being administered three times. The doses of ACh were adjusted according to the amount of depression produced by the antagonist, but they were all correspondingly higher than those used to plot the curve for active drug alone, thus causing a shift of the second curve along the log dose abscissa. The maximal height of contraction in the presence of antagonist was determined by stepping up the dose of ACh until a further increase produced no difference in the height of contraction. It was not always practicable to administer each of these higher doses three times, due to the paralysing effect of repeated higher doses of ACh upon the muscle. As before, the muscle was allowed to recover from the effect of the higher doses before further tests were carried out. All stimulant solutions used to obtain data for the second curve contained the test concentration of antagonist.

The heights of contraction were measured in millimetres, and the mean height at each dose level was computed and expressed as a percentage of the maximal height in the absence of the antagonist. For convenience in plotting, the doses of stimulant were multiplied by one hundred before conversion to logarithmic form. They were plotted as abscissae against the corresponding percentage effects as ordinates.

Test for Parallelism.—To apply a statistical test for parallelism to the concentration-action curves, it was necessary to convert the curves to linear form.

To explain the parallelism frequently observed with many antagonist systems, Gaddum (1937) derived the equation:

$$K_1C_1=(1+K_2C_2^n)\cdot\frac{y}{100-y}$$

where C_1 is the concentration of active drug, C_2 the concentration of antagonist, and y=the effect of the active drug.

From this equation:

$$C_1=\frac{1+K_2C_2^n}{K_1}\cdot\frac{y}{100-y}$$

whence

$$\log_{10}C_1=\left[\log_{10}\frac{1+K_2C_2^n}{K_1}\right]+\left[\log_{10}\frac{y}{100-y}\right]$$

A plot of $\log_{10}C_1$ against $\log_{10}\frac{y}{100-y}$ should therefore be linear. This appeared to be so for all the concentration-action curves obtained. The significance levels of the calculated linear regressions were found to be less than 0.20 for all curves (see Table IV).

The significance of the absence of parallelism was calculated by an analysis of variance.

Composition of Tyrode Solution.—NaCl, 8.0 g.; KCl, 0.2 g.; $CaCl_2$, 0.2 g.; $MgCl_2$, 0.01 g.; NaH_2PO_4, 0.05 g.; $NaHCO_3$, 1.0 g.; dextrose, 1.0 g.; distilled water to 1 litre.

Treatment of Glassware.—Discrepancies in the experimental results due to adsorption of the basic antihistamines on to the glassware (Marshall, 1955a) were avoided by thorough washing in hot tap water followed by immersion in 33% v/v commercial nitric acid overnight; further treatment with hot tap water and distilled water preceded subsequent use. The glassware of the automatic assay apparatus was treated in the same manner.

Results

Tables I and II contain the results of pA_2 and pA_{10} determinations on two series of compounds which are relatively specific antihistamines. The probability (P) was calculated that the differences between (pA_2–pA_{10}) values for two compounds A and B could have occurred by chance. Values of t and P for the halogenated members of two series and their respective non-halogenated "parent" compounds are shown in Table III; values of t were calculated from the formula

$$t=\pm\frac{(pA_2-pA_{10})_A-(pA_2-pA_{10})_B}{\sqrt{(eA_2)^2+(eA_{10})^2+(eB_2)^2+(eB_{10})^2}}$$

in which eA_2 is the standard error of the mean pA_2 for compound A, and eA_{10} is the standard error of the mean pA_{10} for this compound; eB_2 and eB_{10} are the corresponding values for the second compound, B.

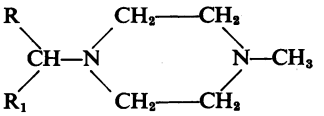
Piperazine Derivatives.—This series provides an interesting illustration of the extent to which relatively small variations of chemical structure can affect pharmacological activity. Table I shows the composition and pA_x values for the members of this series. Compounds A2285, A2342, chlorcyclizine and A2824 differ from the parent compound only in the possession of a halogen atom.

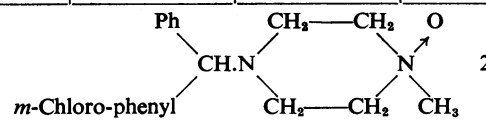
TABLE I

STRUCTURES AND ACTIVITIES OF PIPERAZINE DERIVATIVES

Antagonism to acetylcholine expressed as pA_x values (standard deviations in parentheses; all values are means of four determinations)

General formula:



Serial No.	R	R_1	Salt	pA_2	pA_{10}	pA_2–pA_{10}
A1198 ..	Phenyl	Phenyl	HCl	6·40 (0·03)	5·79 (0·20)	0·61
A2285 ..	*p*-Bromo-phenyl	,,	,,	5·89 (0·09)	5·54 (0·19)	0·35
A2342 ..	*p*-Fluoro-phenyl	,,	,,	6·08 (0·05)	5·62 (0·12)	0·46
Chlorcyclizine	*p*-Chloro-phenyl	,,	,,	5·86 (0·08)	5·25 (0·17)	0·61
A2824 ..	*m*-Chloro-phenyl	,,	,,	6·46 (0·12)	5·72 (0·11)	0·74
A2917 ..	*p*- ,, ,,	2-Thiophen	$2(COOH)_2$	5·86 (0·09)	5·36 (0·16)	0·50
A4826 ..			$2(COOH)_2$ Anti-acetylcholine activity negligible			
A2688 ..	*p*-Methyl-phenyl	Phenyl	HCl	5·75 (0·09)	5·40 (0·11)	0·35

In the first three halogenated compounds, the halogen is in the para position, in the fourth in the meta position of one phenyl ring. The pA_2 values of all three para-halogenated compounds are distinctly lower than that of the parent compound, and only the fourth (meta-halogenated) compound has pA_2 of the same order as that for the parent substance.

Para-methylation of one phenyl ring (compound A2688) appears also to lower the pA_2 value compared with the parent compound, which suggests that this effect is not confined to halogenation. Statistical analysis of the results has shown that the differences in pA_2 and pA_{10} values of halogenated and non-halogenated compounds are not always significant. The pA_{10} values for A2285 and A2342 are not significantly different from that of A1198. With these exceptions, however, halogenation does appear to alter anti-ACh activity in this series, to an extent considered to warrant further investigation.

(pA_2–pA_{10}) *Values.*—(pA_2–pA_{10}) values for these antihistamines are interesting in that they are all below 0.95, the theoretically-derived value for first order competitive antagonists (Schild, 1947). With the exception of chlorcyclizine, the para halogenated compounds showed smaller (pA_2–pA_{10}) differences than the parent compound, though statistically such differences were not always significant (Table III). The compound with the halogen in the meta position (A2824), on the other hand, shows a (pA_2–pA_{10}) difference of the same order as that for the parent substance. The very low (pA_2–pA_{10}) values shown by several of these compounds are considered to indicate a direct depressant effect upon the muscle. There is some evidence for this view. Papaverine, a substance known to depress the muscle directly, was found to have pA_2 of 5.12 and pA_{10} of 5.17 giving the extremely small value for (pA_2–pA_{10}) of 0.05.

These results indicated that there might be an essential difference in the mode of action of the halogenated compounds compared with that of the parent substance.

A further interesting result is shown in Table I. Compound A4826 differs from the parent compound A1198 in two respects. Firstly, A4826 possesses a meta-chlorine atom in one phenyl ring of the benzhydryl moiety, and secondly, the nitrogen atom in the piperazine structure of this compound is linked co-ordinately to an oxygen atom, whereas, in the parent compound, the electron pair of the corresponding nitrogen atom remains free. A4826 had negligible anti-acetylcholine activity. A concentration corresponding to two hundred times that of the pA_2 for the parent compound had only slight activity against the double dose of ACh. (0.025 μg./ml.) The inference is, then, that the tertiary nitrogen atom of the piperazine structure in these compounds is essential to anti-acetylcholine activity. Fixation of the free electron pair from this atom in the formation of a co-ordinate bond (as in A4826), is

TABLE II

STRUCTURES AND ACTIVITIES OF *N*-DIMETHYL-*N*(*n*-PROPYL) AMINE DERIVATIVES

Antagonism to acetylcholine expressed as pA_x values (standard deviations in parentheses; all values are means of four determinations)

Compound	R_1	R_2	pA_2	pA_{10}	pA_2–pA_{10}
Prophenpyridamine ("Trimeton" Schering Corp.)	Phenyl	2-Pyridyl	5·54 (0·03)	4·61 (0·15)	0·93
Chlorprophenpyridamine ("Chlortrimeton," Schering Corp.)	*p*-Chloro-phenyl	,,	5·59 (0·13)	4·99 (0·08)	0·60

accompanied by considerable decrease of anti-acetylcholine activity.

Prophenpyridamine and Chlorprophenpyridamine.—Although the pA_2 values of these compounds were approximately of the same order, the pA_{10} values and consequently the (pA_2–pA_{10}) differences were found to differ significantly (Tables II and III). The (pA_2–pA_{10}) difference of the parent compound was 0.93, the value for a first order competitive antagonist, whereas the value for chlorprophenpyridamine was only 0.60, again an indication that the halogenated member acts in a different manner from the non-halogenated compound.

TABLE III

STATISTICAL TREATMENT OF RESULTS TO TEST EFFECT OF HALOGENATION ON (pA_2–pA_{10})

	Difference Between (pA_2–pA_{10}) for A1198 and Designated Compound	Degrees of Freedom *n*	*t*	*P*
A2285 ..	0·26	12	1·5	$0·1<P<0·2$
A2342 ..	0·15	12	1·25	$0·2<P<0·3$
Chlorcyclizine	0·00	12	—	—
A2824 ..	0·13	12	1·02	$0·3<P<0·4$

Difference Between (pA_2–pA_{10}) for Prophenpyridamine and Chlorprophenpyridamine	Degrees of Freedom *n*	*t*	*P*
0·33	12	3·27	$<0·01$

The results for these two groups of antihistamines indicated that there might be an essential difference in the mode of action of the halogenated compounds compared with that of the parent compound, and suggested the necessity for further investigation.

Concentration-action Curves

Gaddum's formula (1937) for drug antagonism is based on the experimental fact that concentration-action curves are frequently parallel when plotted first in the absence and then in the presence of a constant concentration of antagonist. This fact is also the basis of the *p*A measure, for parallelism on a log dose abscissa indicates that *p*A is independent of the height of contraction, and therefore of the initial concentration of active drug (Schild, 1949). In addition, concentration-action curves may be used as a test of competitiveness in active-drug-antagonist systems (Van Maanen, 1950); if the curves in the presence and absence of the antagonist are parallel, and if the same maximal height of contraction can be obtained in either case, the antagonist is likely to be acting by a competitive mechanism.

It was considered necessary, therefore, to plot concentration-action curves for acetylcholine on the isolated guinea-pig ileum, with the halogenated and non-halogenated "parent" compounds of the two series used in this investigation (Figs. 2–8). By this means, evidence could be obtained concerning the extent to which the *p*A method is applicable to antihistamine/acetylcholine systems, and also the mode of action of these substances when antagonizing ACh.

A preliminary experiment was carried out with atropine and ACh in order to determine what type of concentration-action curve this highly specific antagonist would produce.

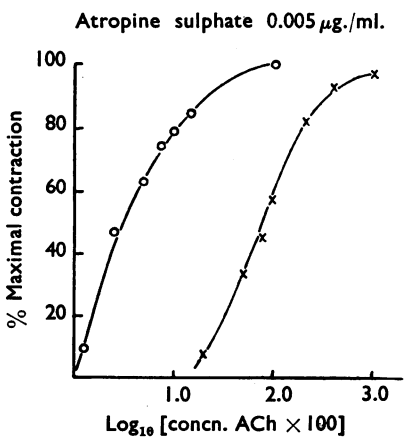


FIG. 1.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine in the presence of atropine sulphate 0.005 μg./ml.

Fig. 1 shows the curves obtained. The second was plotted after equilibration to 0.005 μg. atropine/ml. When analysed statistically the curves showed no significant deviation from parallelism (Table IV) and the effect of atropine was reversible. It is concluded, therefore, that the antagonism is competitive.

TABLE IV

STATISTICAL TEST FOR PARALLELISM ON CONCENTRATION-ACTION CURVES

Antagonist	Significance of Regression for ACh Alone	Significance of Regression for ACh Plus Antagonist	Significance of Absence of Parallelism	Parallel + Non-Parallel
Atropine ..	0 05	<0·001	>0·20	+
Prophenpyridamine ..	<0·01	<0·01	>0·20	+
Chlorprophenpyridamine..	<0·001	<0·05	<0·001	−
A1198 ..	<0·20	<0·001	>0·20	+
A2285 ..	<0·01	<0·50	<0 01	−
A2342 ..	0·001	<0·20	<0·05	−
A2824 ..	<0·01	<0·20	<0·20	−
Chlorcyclizine	<0 01	<0·20	<0·05	−

Antihistamines

It was found that the concentration-action curves for A1198 and prophenpyridamine, the non-halogenated parent compounds, were parallel, and that the effect of the antagonists could be reversed by suitably increasing the ACh concentration (Figs. 2 and 7). The conclusions which may be drawn are, firstly, that these compounds are competitive antagonists of ACh on this preparation, and, secondly, that *p*A is independent of the height of contraction.

The halogenated antihistamines (Figs. 3, 4, 5, 6, 8), on the other hand, produced an effect upon the muscle which could not be reversed by ACh. It may therefore be concluded that their action is non-competitive on this preparation. Furthermore, the pA_x values recorded for these compounds, and particularly the pA_{10} values, were subject to errors arising from the dependence of *p*A upon the height of contraction.

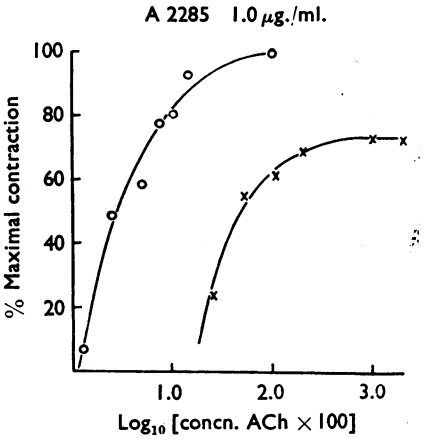


FIG. 3.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to A2285, 1.0 μg./ml.

The curves for the halogenated compounds were not of the type required by the mass law equation derived by Schild (1954) for non-competitive antagonism, which requires that the slopes and maxima of the curves should fall off with increasing concentrations of antagonist.

It was considered that the depression of the maximal response shown with these compounds

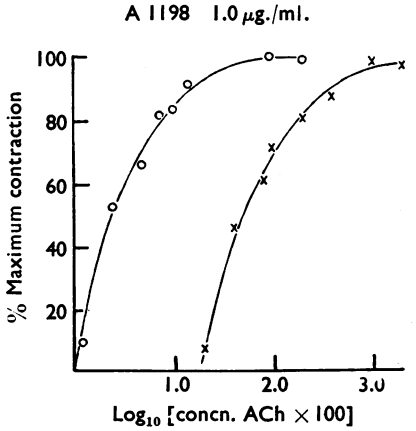


FIG. 2.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to A1198 1.0 μg./ml.

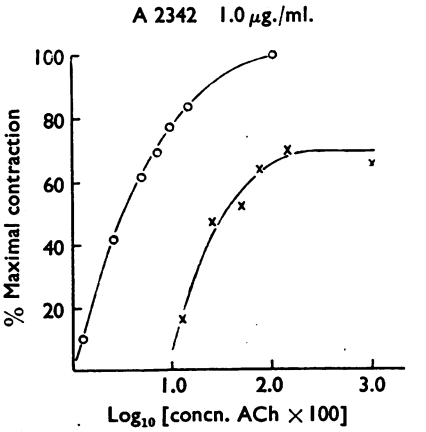


FIG. 4.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to A2342, 1.0 μg./ml.

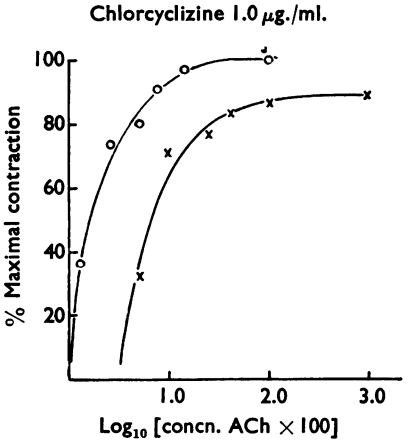


FIG. 5.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to chlorcyclizine, 1.0 μg./ml.

might be due to the high concentrations used, and that lower ones might not show this effect. An investigation of the effects of lower concentrations of antagonists was therefore necessary. It seemed reasonable to suppose that a direct depression of the muscle would be apparent whatever the spasmogen, and hence, in experiments in which the concentration of antihistamine was insufficient to affect responses to ACh, histamine was used as the spasmogen.

Fig. 9 shows the concentration-action curve for chlorcyclizine at a concentration of 10 ng./ml., and Fig. 10 that for 100 ng./ml., against histamine. Figs. 11 and 5 show the corresponding curves for concentrations of 100 ng./ml. and of 1.0 μg./ml.

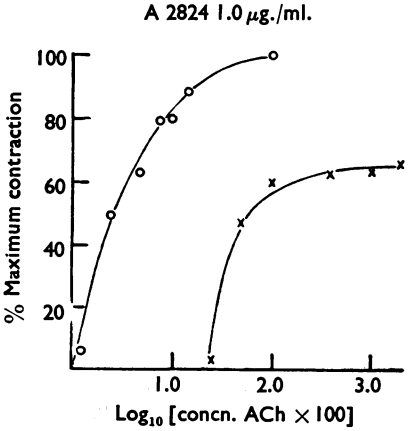


FIG. 6.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to A2824, 1.0 μg./ml.

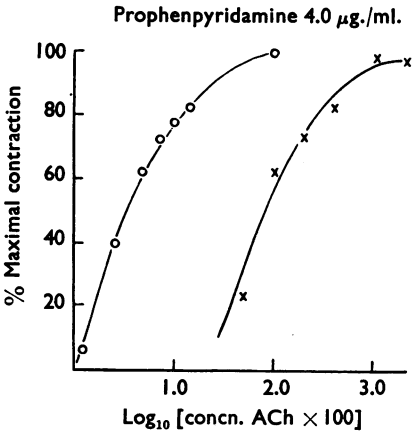


FIG. 7.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to prophenpyridamine, 4.0 μg./ml.

against ACh. The curves show that at concentrations of 10 ng./ml. and 100 ng./ml. there is no direct depression of the muscle, and that a further increase of the chlorcyclizine concentration to 1.0 μg./ml. produces this effect.

DISCUSSION

Non-specific Antagonism

The results presented show that antagonisms of a non-specific nature, such as those between ACh and antihistamines, may be competitive or non-competitive. This depends firstly upon the structure of the antagonist, and secondly upon the relationship between the concentration of antagonist required to produce a reasonable degree of antagonism, and the minimum concentration at which side effects,

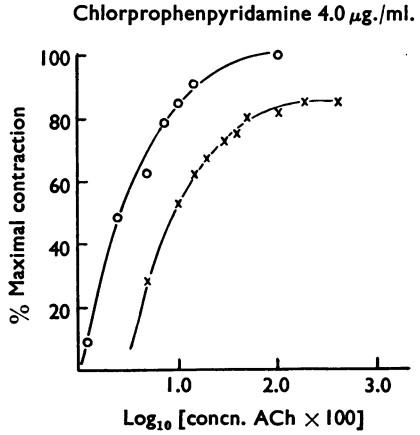


FIG. 8.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to chlorprophenpyridamine, 4.0 μg./ml.

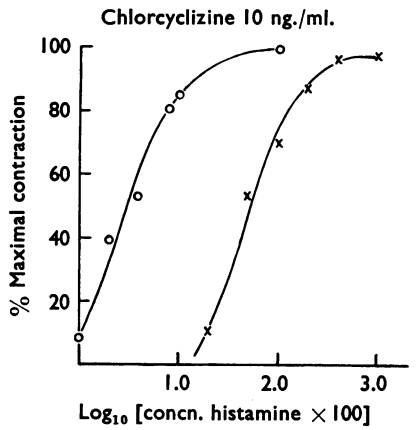


FIG. 9.—Concentration-action curves for histamine on the isolated ileum of the guinea-pig. O——O curve for histamine alone. ×——× curve for histamine after equilibration to chlorcyclizine, 10 ng./ml.

such as non-specific depression of the muscle, occur.

Marshall (1955a) obtained (pA_2–pA_{10}) values greater than 0.95 for some antihistamines against histamine on the guinea-pig ileum. These were considered to be due to "wastage" upon non-specific receptors, such as those for ACh. With higher concentrations of antagonists, such as those required for pA_{10} measurements, it was supposed that so much antagonist was adsorbed upon ACh receptors, that the effective concentration of antagonist in the region of the histamine receptors was reduced. Consequently, a greater increase in the concentration of antagonist was required than that theoretically expected for "competitive" antagonists between pA_2 and pA_{10}. In an attempt to confirm this view, Marshall (1955a) determined

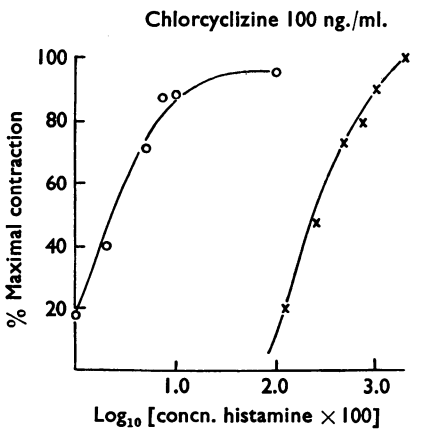


FIG. 10.—Concentration-action curves for histamine on the isolated terminal ileum of the guinea-pig. O——O curve for histamine alone. ×——× curve for histamine after equilibration to chlorcyclizine, 100 ng./ml.

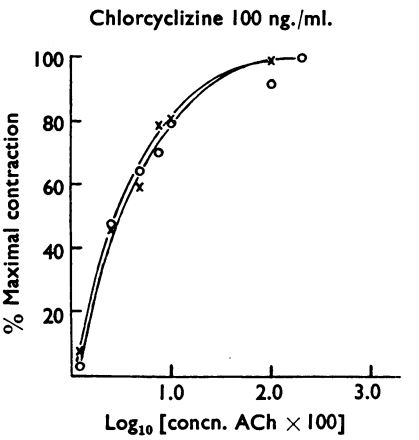


FIG. 11.—Concentration-action curves for acetylcholine on the isolated terminal ileum of the guinea-pig. O——O curve for acetylcholine alone. ×——× curve for acetylcholine after equilibration to chlorcyclizine, 100 ng./ml.

(pA_2–pA_{10}) against histamine for some of the antihistamines which had shown "abnormally high" values, using atropine sulphate in the bathing fluid in order to block the ACh receptors and prevent the access of the antihistamines to these receptors. Only with chlorcyclizine was the expected effect of lowering (pA_2–pA_{10}) to a value nearer 0.95 achieved. In all cases, the actual values of pA_2 and pA_{10} were lowered, some by as much as 0.46 pA units. This observation seems to suggest that, when atropine blocks the ACh receptors, the antihistamines are less effective antagonists of histamine. This is contrary to the concept that fewer antihistamine molecules are then being "wasted" upon ACh receptors.

In this connexion, the experiments described in this paper with a range of concentrations of chlorcyclizine against histamine and acetylcholine are interesting (Figs. 9–11). With 100 ng. chlorcyclizine/ml. (Fig. 10) a hundred-fold increase of histamine was necessary to reproduce a response equal to 50% of the maximum. Even at this high concentration of chlorcyclizine, there was no demonstrable antagonism to ACh (Fig. 11). From this, it may be inferred that there was no significant "wastage" of chlorcyclizine molecules on to ACh receptors.

Marshall's hypothesis is open to criticism on theoretical grounds also. If one assumes a pseudo-monomolecular reaction between the receptors and active-drug molecules on the one hand and antagonist molecules on the other, one must also assume that the effective concentration of receptors is relatively small (Van Maanen, 1950). Hence, the combination of either drug with the receptors would not alter the effective concentration of these substances in the extra-receptor fluid. Accepting this,

it is difficult to see how adsorption of antihistamine molecules upon ACh receptors can affect their concentration and adsorption at the histamine receptors, especially since it has been shown that with chlorcyclizine the amount " wasted " on to ACh receptors is insufficient to affect the responses to ACh.

Values of (pA_2–pA_{10}) significantly less than 0.95 were considered by Marshall (1955b) to indicate non-competitive antagonism. However, it has been shown in this paper that antagonists with (pA_2–pA_{10}) other than 0.95, such as A1198 and atropine, may still give parallel curves. Marshall obtained a (pA_2–pA_{10}) value of 0.73 for atropine sulphate against acetylcholine on the isolated guinea-pig ileum, and concluded that the antagonism was non-competitive. The concentration action curves for acetylcholine with and without atropine (Fig. 1) are parallel and there is no depression of the maximum, suggesting that the antagonism is competitive. The same applies to A1198 and ACh (Table I and Fig. 2). Hence, it cannot be concluded that antagonists for which (pA_2–pA_{10}) is significantly less than 0.95 are non-competitive. In fact, with antagonist/agonist systems to which the pA method has been shown to be applicable by concentration-action curves, (pA_2–pA_{10}) gives only an indication of " n " in the Gaddum (1937) equation, " n " being the number of molecules of antagonist required effectively to block one receptor. Where very low (pA_2–pA_{10}) values are obtained, as with papaverine in the present work, it seems unlikely on logical grounds that a competitive antagonism exists.

It would seem reasonable to expect that one molecule of atropine is capable of blocking one ACh receptor to the exclusion of one molecule of ACh. The failure of atropine to produce values consistent with this hypothesis may either be due to the fact that we are not in fact dealing with a first order reaction, or, as is at least equally probable, the discrepancy may be due to the failure of the Gaddum (1937) equation to express the antagonism exactly. In the absence of any *a priori* reason to suppose that active and antagonistic drugs are reacting chemically together, or that the antagonistic drug alone is capable of exerting a physiological effect which is directly opposed to that of the active drug, the concentration-action curve method used in this work has some value in helping to decide whether an antagonism is competitive or not.

Effects of Halogenation

The present work has revealed the interesting fact that relatively small alterations in structure, such as para-halogenation, have the ability to alter not only the potency of an antagonistic drug but also its mode of action. This has been shown for two series of antihistamines, when tested for their ability to antagonize the ACh-induced spasm of the isolated guinea-pig ileum. With the piperazine antihistamines, para-halogenation, as in compounds A2285, A2342, and chlorcyclizine, appeared to alter the potency of the antagonists more than meta-halogenation, as in A2824, but both substitutions altered the mode of action of the antagonists as shown by the concentration-action curves. The concentration-action curves for halogenated antihistamines of both series provide two pieces of information. In the first place the pA measurements are not reliable, particularly the pA_{10} values, where higher concentrations of antagonists are necessary. Secondly, in distinction to the non-halogenated " parent " compounds, prophenpyridamine and A1198, which are competitive antagonists of ACh, the halogenated members are non-competitive. The concentration-action curves for these compounds are different from those described by Zadina (1947) and Zadina and Kriz (1948) and also from those described by Guarino and Bovet (1949), and must therefore represent a different type of antagonism.

The depressed maximal response to ACh seen with the halogenated antihistamines studied in this work might be explained in three ways. Firstly, the halogenated compounds might act irreversibly upon some of the higher threshold ACh receptors. Their effect would then be the inactivation of some of these receptors. This explanation would still hold good even if only 50% of the receptors were occupied by ACh during a maximal contraction. The second possibility is that the halogenated antihistamines depress the muscle directly and not by a receptor mechanism. The manner in which halogenation produces this effect could possibly be that the toxicity of the compounds to the smooth muscle is increased by the introduction of an electronegative atom into the structure, which lowers the threshold concentration for direct depression of the muscle. Finally, there remains the possibility that the halogenated compounds, but not the non-halogenated compounds, are able to antagonize the ganglionic component of the contractions of the guinea-pig ileum in response to ACh (Feldberg, 1950). This aspect of their mode of action has yet to be investigated.

Effects of Methylation

It is possible that the effects ascribed to halogenation may be produced by other substitutions

as well. For example, the introduction of a methyl group into the para-position of one phenyl ring in the piperazine derivatives as in A2688, had the effect of lowering the (pA_2–pA_{10}) difference for the compound compared with the " parent " compound, a similar effect to that produced by para-halogenation. However, the analogy between the effects of these two substitutions can be taken no further in the absence of additional evidence upon the mode of action of the methylated compound.

A4826.—An interesting point in structure-activity relationships is brought out by the results obtained with this compound. The incorporation of the lone electron pair of one of the piperazine nitrogen atoms into a co-ordinate linkage with an oxygen atom (Table I) results in an almost complete loss of anti-ACh activity. This suggests that the free electron pairs of the piperazine nitrogen atoms must remain free in order that the compounds may exhibit anti-ACh activity.

Summary

1. The activity of two series of antihistamines against the ACh-induced spasm of the isolated guinea-pig ileum were investigated by the *p*A method. In both series of antihistamines, the introduction of a halogen atom in the para-position of one phenyl ring had a marked effect upon the activity of the compounds as ACh antagonists. Meta-halogenation had less effect. There is evidence that methylation may have similar effects.

2. The modes of action of these substances and the validity of the *p*A measurements were investigated by plotting concentration-action curves for ACh in the absence and then in the presence of the antagonists. It is concluded that the introduction of halogen atoms into these two types of compound produces non-competitive antagonists of ACh, their non-halogenated counterparts being competitive.

3. Atropine is probably a competitive antagonist of ACh on the isolated guinea-pig ileum.

4. Criteria by which the mode of action of antagonists may be judged are discussed.

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